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64 bp **↔** 147 bp mmaA2 mmaA1 mmaA4 mmaA3 D 861 bp 864 bp 906 bp 882 bp

166 bp

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Figure 1: Schematic diagram of methoxy mycolic acid synthase mmaA 4-mmaA 1 gene cluster of mycobacteria and location of forward A, and reverse D primers.

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rcaaccatttcaatcgtcgcaggttggcccccgggaagatttcggtcgcgatgaacttgagaaagcgggccagcc ATGTACCGCTCGTAGACCTCTTCGGACTGGATCGCGATGGCCTCGCTTTTTGTGTTCCTGCAGCGCCTCGGCCCACAG CCTAGCTTGCCCAGCGCCAGGTCGATCTTGGCGATCTGGGCCTCTTCCAGCGTCATGTCCTCGCGTTCGAAATGCGC TCTTCGGACTGTACGGCGATGGCGCGTTCGCGGGCAGCCTGTAGGTTGGCGGCCCATGCATC*GAGAGICCGTGCGTA* TCTTTGCTGCGTTCATAGTGATTCCGGCTGAGCGTGAGGCCGATGACATTGACGTCGTACTTCTCCACGGCCCGAAC CTACTTCGCCAGCGTGAACTGGTTGACGTCGATGTAGCCGACCCGGAACAGCTTGGCGCAGCCGGTCAGGTATTTC GTCGAGGGTCCTGGCGTAATGCGGCTGCAGCGACTGGCGGCGAGTCAGCGTGAAACCCGTCTTCGCCGACTGTTCC CATCGGGCGGCAGGATTTTGTGGGCCCGGGCGAAGAAGTCGGCGTGACGATCGTGGCCGAAGTGCTCGAACGCGC CGATCGACACGATGCGGTCGACGGGCTCGTTGAACTGCTCCCATCCCGCCAGCAACACTCGCCTGTCGCGGGGGT gcatgctcgaggcatgctatccgatacagggccgcactaaaccgcgatcgaatttgcccaggtcagggaacggatatgagcggacgacgacgaCTACTTGGTCATGGTGAA CTGGGCGACGTTGATTAGGCCTCTGCGGAAGCGCTCCGCGCATCCGGTCAGATAGTGCATGAAGTTGTTAGAGC <u>GIG</u>CTGCTGCAGCAGCTGGACATGCTCGATGGTGAAGCCCGCGGCCTGCGCATTGTCGACAATGTCGGGCTCCGAT GAGCGCCCCCCCCCCCCCCACCCACGTCGAGTAGCGTCATCCCCGGTTCGAGGTTCAGCTTGTCCAACGCCAGA CCCTGTTCGTGCCACCTGCGGTCGTAGGTGAACAGGCTGTGCAGTAGCATCCGCCCGTCATCGGGCAGGATGT CGTAGGAGCGTTCGAAGAACGTCAGATACCGCTCCTTTTTGAACGCGTCGAATGCCTCAAAGCTGACGATCCGGTC GATCGAGGAACAACGCGAAGAAGTCATCCGAAATGTCGTAAGCCGACTGTGACTCTTCGTAATATGGTCTCAGCTT ACAACGTGAGCGGCAAGCCGTGGTCGACCATCTGCTGCTGGTCAGGCCGGTGATCGTGTGCAGCAGCACACGC GTCCATCTCGACGACGACTTCTGCACATGGGCGGCCTGGTTCTTCGACAATGTCAGGCCGACGACGTTGACGTCA IACTGCGCGATCGCGCCGCGTGGTGGCCCCCAGCCGCAACCGATATCGAGCAGCGTCATGCCGGGCTGCAGA GCAGCTGTAGGTCTGGGTCCGGATCCAGGAACAGCCGGAAGAAGAGTCGTCGGACAGGTCGTAGTGTGCCTGCACGTC CTCGAAGTGCGGCGTTAGGTCGTTGACCATgaggtgtaatgcctttccggaccctaggtggcctttcggtgcttgcacggaacgcaccgatgcttcccctcccc GGCAGCTCGCCCCCGGGAAGATCGACTCCCGCAGGAATTTGAGGAATCGAAGGTCGCTCATCGTCAGCGCAATG TCCACCTTGGCCAGTTGCGCCTCTTCCAGCGTCATATCGTCACGCTCGAAATAGGCGCAGGTGTAGACCCAGGTGG GACGTTCTCTTCAAACTCTTCCCAGCCCTGCAGCCGGGCCTCGGCGCGCCGTTGCGTTCCGATTGCGGCCAGGCGG

Fig. 2: Sequence of mmaA2 and mmaA1 gene with an intergenic region of 166 base pair (shown in lower case. Location of forward A, sequence ID 1 and reverse primer D, sequence ID 2. Both primer sequence is underlined and italisized.



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1 2 3 4 5 6 7 8 9 10 11 12 13 14

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Fig. 3 PCR amplification of different mycobacterial genomic DNAs with primers A and D (lanes 1- 15): 1. M.avium 2. M.bovis 3. M.chelonae 4. M.fortuitum 5. M.intracellulare 6. M.kansassi 7. M.phlei 8. 100 bp DNA ladder 9. M.marinum 10. M.scrofulaceum 11.M.smegmatis 12. M.szulgai, 13. M.tuberculosis and 14. negative control. AD indicates 363 bp-amplified product.

Spacer 166 bp mmaA1 175 bp

MspI 33 bp

HaeI 47 bp

MspI 244 bp

HaeI 219 bp

Fig. 4: line diagram showing restriction endonuclease map of HaeI and MspI within AD.

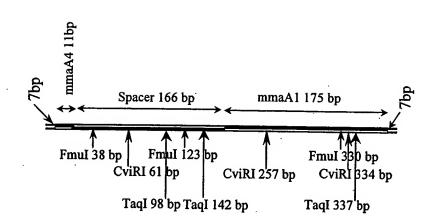


Fig. 5: line diagram showing restriction endonuclease map of Fmul, CviRI and TaqI within AD.

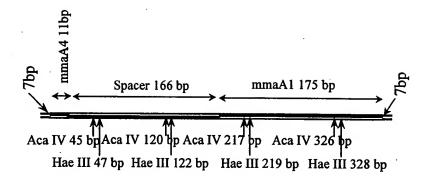


Fig. 6: Restriction map of AD showing distribution of the sites of restriction endonucleases AcaIV and HaeIII.

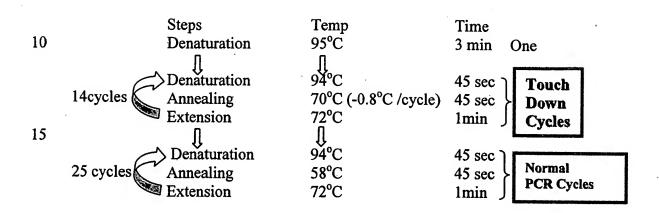


Fig. 7: Line diagram showing different steps of PCR reaction